PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference UNI-004-PCT	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP 03/11192	International filing date *(day/month/year)* 09.10.2003	Priority date *(day/month/year)* 09.10.2002
International Patent Classification (IPC) or both national classification and IPC A61K35/78		
Applicant UNIBIOSCREEN S.A. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

I	☒	Basis of the opinion
II	☐	Priority
III	☒	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain documents cited
VII	☐	Certain defects in the international application
VIII	☐	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
05.05.2004	30.12.2004
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Markopoulos, E Telephone No. +49 89 2399-8658

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/11192

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-50 as originally filed

Claims, Numbers

1-16 received on 08.11.2004 with letter of 05.11.2004

Drawings, Sheets

1/27-27/27 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 15 in respect of industrial applicability

   because:

   ☒ the said international application, or the said claims Nos. 15 relate to the following subject matter which does not require an international preliminary examination (specify):

   see separate sheet

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-16
	No:	Claims	-
Inventive step (IS)	Yes:	Claims	1-16
	No:	Claims	-
Industrial applicability (IA)	Yes:	Claims	1-14,16
	No:	Claims	-

2. Citations and explanations

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/11192

see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claim 15 relates to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of this claim (Article 34(4)(a)(I) PCT).

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Reference is made to the following documents:

D1: SMIT H F ET AL: "AYURVEDIC HERBAL DRUGS WITH POSSIBLE CYTOSTATIC ACTIVITY" JOURNAL OF ETHNOPHARMACOLOGY, ELSEVIER SCIENTIFIC PUBLISHERS LTD, IE, vol. 47, 1995, pages 75-84, XP000885469 ISSN: 0378-8741

D2: DATABASE BIOSIS [Online] BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; 1986, BHATNAGAR S K ET AL: "EFFECTS OF 50 PERCENT ETHANOL EXTRACT OF CALOTROPIS-PROCERA AIT.F. ON ULCERS CAUSED BY ASSORTED TYPES OF CARCINOMA" XP002243509 Database accession no. PREV198784006510

Regarding D2, the whole document has been retrieved and taken into consideration.

2. Novelty

D1 discloses an investigation for Ayurvedic herbal drugs with cytostatic activity. Extracts of the flowers of Calotropis procera as well as nuts of Semecarpus anacardium displayed the strongest cytotoxic effect. The plant material was ground and extracted with 70% ethanol using Soxhlet extraction (p. 79). The cited cardenolides of Calotropis responsible for the effect and described in literature are the following: calotropin, calactin, calotoxin, calotropagenin, proceroside, syriogenine, uscharidin, uscharin, uzarigenin, and voruscharin (p. 82).

D2 discloses the effects of a 50% ethanol extract of Calotropis procera on ulcers caused by various types of carcinoma whereby 60% growth regression could be shown.

Since neither D1 nor D2 disclose neither a combination of Calotropis procera extracts with another compound or with a physical treatment nor the extraction process as claimed in the present application, novelty can be acknowledged for the subject-matter of claims 1-16 in view of D1 and D2.

3. Inventive step

D1 is regarded as being the closest prior art to the subject-matter of claims 1-16.
The subject-matter of claims 1-16 therefore differs from this known prior art in that: Calotropis procera extract is not combined with other compounds or used together with a physical treatment in patients but the extracts are being used for determining cytotoxicity in vitro. Regarding claims 13 and 14, the material is not disolved in alcohol and filtered prior to ethanol Soxhlet extraction in D1.

The problem to be solved by the present invention may therefore be regarded as finding other alternatives in order to prepare an improved anti-cancer drug from extracts of C. procera, especially for reducing side effects.

The solution proposed in claims 1-16 of the present application seems to involve an inventive step (Article 33(3) PCT) for the following reasons.

As D1, D2 shows as well the effects of ethanol extracts of C. procera, but in a clinical study with 23 cancer patients. The skilled man is aware of the ethnobotanical use of this plant and knows the cardenolides being responsible for the effects.
But the combined preparation with other anti-tumour drugs is neither shown nor suggested in the prior art at hand. A synergistic effect has been shown in example 8 of the application where the survival of mice could be significantly prolonged by using a combination of C. procera with two anti-cancer drugs compared to mice treated with the anti-cancer drugs alone.
In regard to the extraction method of claim 13, the applicant submits that it provides another activity to the extract, namely an anti-poisonous activity since the extracts obtained by this method have the ability to reduce toxic side effects of anti-cancer drugs. Such an anti-poisonous activity for the usual Soxhlet extracts is not mentioned in D1 or D2 and would not be obvious for the skilled in the art.

The applicant is informed that since the therapeutic compound and physical treatment in claim 1 are defined in terms of the result to be achieved, without providing the technical features necessary for achieving this result, the matter of inventive step will be reexamined in the national phase.

4. For the assessment of the present claims 1-12 and 15 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Claims

1. Use of a composition comprising an extract of *Calotropis procera*, and at least one therapeutic compound and/or a physical treatment that exerts relevant, detrimental side effects on normal, non-cancer related cells, tissues or organs for the preparation of a medicament for treating cancer.

2. Use of a composition according to claim 1, wherein said extract is obtained using an extraction procedure, comprising the steps of:
   a) extracting the starting material of said *Calotropis procera* plant, said starting material being selected among fruits, aerial parts subterranean parts, and their mixtures, in an aliphatic alcohol, by dissolving the starting material in said alcohol thereby obtaining a suspension of said material in said alcohol, stirring said suspension, and filtering said suspension by fritted glass thereby obtaining a first filtrate and a first solid part;
   b) extracting said first solid part in an aliphatic alcohol thereby obtaining a second filtrate and a second solid part;
   c) combining said first and said second filtrate thereby obtaining a combined filtrate, and evaporating said combined filtrate under vacuum thereby obtaining said extract.

3. Use of a composition according to claim 1 or 2, where said extract comprises at least two active compounds selected from the group comprising asclepin, calactin, vorusharin, calotropin, calotropagenin, uzarigenin, calotoxin, usharin, usharidin, and 2"oxo-vorusharin.

4. Use of a composition according to any of claims 1 to 3, wherein the weight ratio of extract: therapeutic compound is in the range 0.001 : 1 to 1000 : 1.

5. Use of a composition according to any of claims 1 to 4, wherein said cancer is selected from the group comprising breast cancer, lymphoma, sarcoma,

pancreatic cancer, melanoma, colorectal cancer, glioma, non small cell lung cancer, small cell lung cancer, skin cancer, bone cancer, ovarian cancer, CNS cancer, renal cancer, bladder cancer, head and neck cancer, prostate cancer, liver cancer, hematological cancers.

6. Use of a composition according to any of claims 1 to 5, wherein said therapeutic compound(s) is an anti-cancer agent.

7. Use of a composition according to any of claims 1 to 6, wherein said therapeutic compound is selected from the group comprising adriamycin, alkeran, ara-c, bleomycin, biCNU, busulfan, CCNU, carboplatinum, cisplatinum, cyclophosphamide, cytoxan, daunorubicin, DTIC, 5-FU, fludarabine, gemcitabine (gemzar), herceptin, hexamethylmelamine, hydrea, idarubicin, ifosfamide, irinotecan (camptosar, CPT-11), leustatin, methotrexate, mithramycin, mitomycin, mitoxantrone, muphoran, navelbine, nitrogen mustard, oxaliplatine, rituxan, STI-571, streptozocine, taxol, taxotere, topotecan (hycamtin), velban, vincristine, VP-16, xeloda (capecitabine), or zevelin.

8. Use of a composition according to any of claims 1 to 6, wherein said therapeutic compound(s) is a cytotoxic antibody or a fragment thereof.

9. Use of a composition according to any of claims 1 to 6, wherein said therapeutic compound(s) is a cytotoxic hormone or a fragment thereof.

10. Use of a composition according to any of claims 1 to 6, wherein said therapeutic compound(s) is a cytotoxic peptide or a fragment thereof.

11. Use of a composition according to any of claims 1 to 5, wherein said therapeutic compound(s) is therapeutic radiation.

12. Use of a composition according to any of claims 1 to 11, wherein said extract is administered prior to, after, or at the same time as said therapeutic compound(s).

13. An extraction process for obtaining an extract having biologically active components comprising the steps of:

a) extracting the starting material of said *Calotropis procera* plant, said starting material being selected among fruits, aerial parts, subterranean parts, and their mixtures, in an aliphatic alcohol, by dissolving the starting material in said alcohol thereby obtaining a suspension of said material in said alcohol, stirring said suspension; and filtering said suspension by fritted glass thereby obtaining a first filtrate and a first solid part;

b) extracting said first solid part in an aliphatic alcohol thereby obtaining a second filtrate and a second solid part;

c) combining said first and said second filtrate thereby obtaining a combined filtrate; and

d) evaporating said combined filtrate under vacuum thereby obtaining said extract.

14. Active extract isolated from the process according to claim 13.

15. A method for treating cancer comprising administering to an individual in need of such treatment a pharmaceutical composition as defined in any of claims 1 to 12.

16. A kit comprising a container in which an extract of *Calotropis procera* a as defined in any of claims 1 to 12 is present, and a container in which a therapeutic compound is present.